The VAB-1 Eph Receptor Tyrosine Kinase Functions in Neural and Epithelial Morphogenesis in C. elegans

Sean E. George,§ Kristin Simokat,† Jeff Hardin,‡ and Andrew D. Chisholm§

*Department of Biology
Sinsheimer Laboratories
University of California
Santa Cruz, California 95064
†Program in Cellular and Molecular Biology
‡Department of Zoology
University of Wisconsin
Madison, Wisconsin 53706

Summary

Mutations in the C. elegans vab-1 gene disrupt the coordinated movements of cells during two periods of embryogenesis. vab-1 mutants are defective in the movement of neuroblasts during closure of the ventral gastrulation cleft and in the movements of epidermal cells during ventral enclosure of the embryo by the epidermis. We show that vab-1 encodes a receptor tyrosine kinase of the Eph family. Disruption of the kinase domain of VAB-1 causes weak mutant phenotypes, indicating that VAB-1 may have both kinase-dependent and kinase-independent activities. VAB-1 is expressed in neurons during epidermal enclosure and is required in these cells for normal epidermal morphogenesis, demonstrating that cell-cell interactions are required between neurons and epidermal cells for epidermal morphogenesis.

Introduction

The shape of an organism is determined by the morphogenetic behavior of its cells. While we know much about the process of morphogenesis at the descriptive level and from experimental embryology and biochemistry, relatively little is known of the genetic mechanisms underlying morphogenetic movements (Bard, 1992). Many common types of morphogenetic movements involve epithelia, such as invagination and spreading (epiboly). One approach to understanding the molecular mechanisms underlying such movements is to use genetic analysis to identify mutants defective in such movements. Genetic analysis in Drosophila melanogaster has identified cell signaling pathways required for dorsal epidermal closure (Knust, 1997), including a JNK pathway that regulates cell shape changes in the leading edge (Glise et al., 1995; Riesgo-Escovar et al., 1996; Sluss et al., 1996), and TGFβ signaling pathways that may transmit signals to cells behind the leading edge. Another type of epithelial morphogenesis that has been analyzed genetically is ventral furrow invagination during Drosophila gastrulation. Genetic screens have identified two loci required for cell shape changes in gastrulation: the β subunit concertina (cta) (Parks and Wieschaus, 1991) and the novel secreted protein folded gastrulation (fog) (Costa et al., 1994). Thus, both these types of epithelial morphogenesis involve cell signaling, either via J NK and TGFβ pathways in epiboly or via cta and fog pathways in invagination.

The epidermis of the nematode Caenorhabditis elegans is a simple system in which to analyze epithelial morphogenesis. The epidermis, also known as hypodermis, begins as a set of cells born in the dorsal part of the embryo (Sulston et al., 1983) that forms an epithelial sheet. Ventral enclosure of the embryo by the epidermis occurs in two steps (Williams-Masson et al., 1997). Four anterior epidermal cells lead the migration of the epidermis to the ventral midline by extending actin-rich filopodia over substrate neurons. Once these leading cells have reached the midline, the remainder of the ventral midline appears to be enclosed by an actin-mediated purse-string mechanism. After enclosure is complete, circumferential constriction in the epidermis squeezes the embryo longitudinally (Priess and Hirsh, 1986).

To identify genes involved in epithelial morphogenesis, we have analyzed mutants in which morphogenesis is defective. vab-1 mutants were originally isolated by Brenner (1974) based on their morphogenetic defects in head epidermis. Here, we show that vab-1 is also required for cell movements following gastrulation and during ventral closure of the epidermis. We show that vab-1 encodes an Eph receptor expressed in neuroblasts and neuronal cells and that vab-1 function in these neuronal cells is required for epidermal morphogenesis. Our results provide an example of interactions between neuronal substrate cells and a migrating epithelial sheet in morphogenesis.

Results

vab-1 Mutants are Defective in Morphogenesis

The most striking defect of vab-1 mutant larvae is the deformation of head epidermis, the “Notched head” phenotype (Brenner, 1974; Figures 1A and 1B); epidermal morphogenesis is often also abnormal in the tail region (Figure 1B). Morphology of the body is normal in most vab-1 mutants. The phenotypes caused by vab-1 alleles are incompletely penetrant and variably expressed, hence the gene name vab (variable abnormal). The number of head epidermal cells and nuclei is normal in Notched head vab-1 mutants, as determined by Nomarski microscopy and expression of epithelial markers (data not shown), indicating that the Notched head phenotype results specifically from abnormal morphogenesis of head epidermal syncytia (Figures 1C and 1D).

Seventeen recessive zygotic vab-1 mutant alleles have been isolated by various workers (see Experimental Procedures). We classified vab-1 alleles as strong, intermediate, or weak, based on the penetrance of mutant phenotypes (Table 1); most animals mutant for strong vab-1 alleles arrest during embryogenesis due to defects in epidermal enclosure (see below), whereas weak vab-1 mutants are almost fully viable. By genetic and molecular criteria (Tables 1 and 2), the strong vab-1 alleles cause complete loss of function.

§To whom correspondence should be addressed.
vab-1 Null Mutations Cause Variable Defects in Cell Movements following Gastrulation and during Embryonic Ventral Enclosure of the Epidermis

To determine the role of vab-1 in embryonic development of the epidermis, we analyzed the embryogenesis of vab-1 mutant embryos using conventional and four-dimensional Nomarski microscopy. We found that vab-1 null mutant embryos are variably defective in the movements of neuroblast cells during closure of the ventral gastrulation cleft and in the migrations of epidermal cells during ventral enclosure of the epidermis. These defects in cell movements result in failure of enclosure of the embryo by epidermal cells, and such embryos arrest because internal cells leak out at the ventral midline.

The variable phenotypes of vab-1 null mutants can be described in terms of five phenotypic classes (Figure 2 legend). Approximately 35% of vab-1 null mutant animals displayed defects in cell movements following gastrulation. During normal C. elegans gastrulation, a ventral cleft is formed by the movement of endoderm, mesoderm, and germline precursors into the interior of the embryo; this cleft is gradually closed by the short-range lateral movements of many neuroblasts (Figures 2A and 2C) (Sulston et al., 1983). In 20% of vab-1 embryos (phenotypic classes I and II), the ventral cleft is deeper than normal during ventral enclosure of the epidermis and remains for longer (Figure 2E); in 15% of embryos the cleft is of normal depth and lasts longer (class III). These phenotypes appear to result from delays in the

Table 1. Strength of vab-1 Mutations and Molecular Lesions

<table>
<thead>
<tr>
<th>Allele</th>
<th>Embryonic Arrest</th>
<th>Larval Arrest</th>
<th>Adult, Vab</th>
<th>Adult, Non-Vab</th>
<th>Wild-Type Sequence</th>
<th>Mutant Sequence</th>
<th>Predicted Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e2027</td>
<td>58.2%</td>
<td>31.3%</td>
<td>8.9%</td>
<td>2.5%</td>
<td>ND</td>
<td>74 bp deletion removing first 7 bp of exon 5</td>
<td></td>
</tr>
<tr>
<td>e721</td>
<td>58.2%</td>
<td>29.3%</td>
<td>11.4%</td>
<td>1.0%</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>dx14</td>
<td>56.1%</td>
<td>29.6%</td>
<td>9.5%</td>
<td>4.7%</td>
<td>—</td>
<td>deletion of exon 4, part of exon 5</td>
<td>E62K</td>
</tr>
<tr>
<td>ju8</td>
<td>53.3%</td>
<td>32.5%</td>
<td>13.2%</td>
<td>0.8%</td>
<td>CAAA</td>
<td>deletion of exons 1-4</td>
<td>Q21amber</td>
</tr>
<tr>
<td>dx31</td>
<td>50.8%</td>
<td>33.2%</td>
<td>14.0%</td>
<td>2.0%</td>
<td>CAG</td>
<td>TAG</td>
<td></td>
</tr>
<tr>
<td>e1059</td>
<td>49.9%</td>
<td>36.0%</td>
<td>12.5%</td>
<td>1.4%</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tn2</td>
<td>30.3%</td>
<td>14.9%</td>
<td>33.4%</td>
<td>21.3%</td>
<td>TGG</td>
<td>TAG</td>
<td>W932amber</td>
</tr>
<tr>
<td>e856</td>
<td>19.9%</td>
<td>26.7%</td>
<td>45.0%</td>
<td>8.4%</td>
<td>GAG</td>
<td>AAG</td>
<td>E195K</td>
</tr>
<tr>
<td>e699</td>
<td>9.0%</td>
<td>20.9%</td>
<td>51.2%</td>
<td>19.8%</td>
<td>ACT</td>
<td>ATT</td>
<td>T63I</td>
</tr>
<tr>
<td>Weak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e118</td>
<td>10.1%</td>
<td>8.6%</td>
<td>45.6%</td>
<td>35.8%</td>
<td>—</td>
<td>326 bp deletion in exon 10</td>
<td></td>
</tr>
<tr>
<td>e2</td>
<td>10.2%</td>
<td>5.5%</td>
<td>31.4%</td>
<td>51.4%</td>
<td>GGA</td>
<td>GAA</td>
<td>G917E</td>
</tr>
<tr>
<td>ju63</td>
<td>9.2%</td>
<td>7.0%</td>
<td>52.1%</td>
<td>31.5%</td>
<td>CAG</td>
<td>TAG</td>
<td>W964amber</td>
</tr>
<tr>
<td>ju22</td>
<td>8.5%</td>
<td>6.4%</td>
<td>53.8%</td>
<td>31.2%</td>
<td>TGT</td>
<td>TTT</td>
<td>C966F</td>
</tr>
<tr>
<td>e1063</td>
<td>8.3%</td>
<td>6.6%</td>
<td>51.0%</td>
<td>34.0%</td>
<td>TGT</td>
<td>TAT</td>
<td>C966Y</td>
</tr>
<tr>
<td>e116</td>
<td>6.4%</td>
<td>17.5%</td>
<td>50.7%</td>
<td>25.3%</td>
<td>TGG</td>
<td>TGA</td>
<td>W921opal</td>
</tr>
<tr>
<td>e200</td>
<td>5.6%</td>
<td>3.1%</td>
<td>44.4%</td>
<td>46.7%</td>
<td>tttcagAAG</td>
<td>tttcaajAAG</td>
<td>exon 2 splice acceptor</td>
</tr>
<tr>
<td>e1029</td>
<td>5.9%</td>
<td>1.9%</td>
<td>45.7%</td>
<td>46.5%</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

Mutant phenotypes were quantitated as described in Experimental Procedures. We classify vab-1 mutations as strong (>50% lethality), intermediate (25%–50% lethality), or weak (<25% lethality). Most larval arrest animals arrested during L1 or L2 stages; some vab-1 larvae rupture at the rectum. Some (<5%) adult animals showed egg-laying defects. Wild-type and mutant vab-1 genomic DNA sequences are shown with the predicted effects on VAB-1 protein.

ND, not determined.
lateral movements of the neuroblasts that normally close the gastrulation cleft. Thus, vab-1 function is required for neuroblast movements following gastrulation.

Approximately 35% of vab-1 null mutant embryos displayed severe defects in cell movements during the process of epidermal enclosure. During ventral enclosure of the epidermis in wild-type embryos, leading cells of the epidermis migrate over neurons, from lateral positions to the ventral midline (Figures 2B and 2D) (Williams-Masson et al., 1997). The epidermal leading edge cells fail to migrate or migrate more slowly than normal in vab-1 animals (phenotypic class I; Figure 2F), or they migrate to the ventral midline and fail to form junctions correctly (class II). The leading cells in class I and II embryos often send actin-rich processes to abnormally anterior regions, as determined by phalloidin staining (data not shown), and migrate to abnormally anterior positions (Figure 2F). As a result, ventral closure is incomplete, and internal cells ooze through a hole in the ventral midline of the epidermis when elongation begins, resulting in rupture and arrest of the embryo. In other embryos, closure of the gastrulation cleft appears normal, and the embryos elongate to the conserved among Eph family members, and two fibronectin type III repeats. The intracellular domain of VAB-1 contains an insert sequence (A719-E743) between subdomains I and II. The range of phenotypes of vab-1(e2027), vab-1(e2027)/Df, and Df/Df animals are similar, consistent with vab-1(e2027) being a null mutation.

### Table 2. The vab-1 Null Phenotype Is a Variable Defect in Morphogenesis

<table>
<thead>
<tr>
<th>Parental Genotype (n)</th>
<th>Arrest at or before 2-Fold</th>
<th>Arrest Later Than 2-Fold</th>
<th>Hatched, Abnormal Head or Tail</th>
<th>Hatched, Normal Head and Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>vab-1(133)</td>
<td>18.0% (24)</td>
<td>23.3% (31)</td>
<td>58.6% (78)</td>
<td>0% (0)</td>
</tr>
<tr>
<td>vab-1/+ (152)</td>
<td>8.5% (13)</td>
<td>7.8% (12)</td>
<td>11.2% (17)</td>
<td>72% (110)</td>
</tr>
<tr>
<td>vab-1/+ x ccDf4/+ (B1)</td>
<td>8.6% (7)</td>
<td>5% (4)</td>
<td>11.1% (9)</td>
<td>75.3% (61)</td>
</tr>
<tr>
<td>ccDf4/+ x madDf4/+ (105)</td>
<td>12.3% (13)</td>
<td>7.6% (8)</td>
<td>3.8% (4)</td>
<td>76.2% (80)</td>
</tr>
</tbody>
</table>

Arrest stages of vab-1 mutants were scored as described in Experimental Procedures. Arrest at or before 2-fold stages corresponds to phenotypic Classes I-III and some Class IV phenotypes, arrest later than 2-fold corresponds to a subset of Class IV phenotypes, embryos that hatch correspond to Class V. Data for the strong vab-1 allele e2027 are shown; similar results were obtained for other strong vab-1 alleles. Similar ranges of phenotypes are seen in progeny from homozygous vab-1(e2027) mothers and in one quarter of the progeny of vab-1(e2027)/+ mothers, indicating that there is no maternal effect for vab-1. The predicted VAB-1 protein (Figure 4A) is most similar to Eph-related RPTKs, recently renamed Eph receptors (Eph Nomenclature Committee, 1997). The Eph receptor subfamily is the largest subfamily of RPTKs (Orioli and Klein, 1997). EphA receptors bind GPI-linked ephrin ligands, and EphB receptors bind transmembrane ephrins (Gale et al., 1996); VAB-1 shows equal sequence similarity to EphA and EphB subclasses. VAB-1 contains all hallmark features of Eph receptors (Figures 3B and 3C), including in its extracellular domain an N-terminal globular domain with weak similarity to immunoglobulin domains (O’Bryan et al., 1991), a cysteine rich domain, in which the positions of the cysteine residues are highly conserved among Eph family members, and two fibronectin type III repeats. The intracellular domain of VAB-1 contains the juxtamembrane motif Y(I/V)DPXTYEDP found in all vertebrate Eph receptors and a tyrosine kinase catalytic domain most similar (59% identical) to that of human EphA3/Hek and between 51% and 58% identical to those of other Eph receptors (Figure 3E). Unlike other Eph receptor kinase domains, the VAB-1 kinase domain contains an insert sequence (A719-E743) between subdomains I and II.

### Mutations in the VAB-1 Extracellular Domain

#### Cause Strong or Intermediate Mutant Phenotypes

To identify functionally important parts of the VAB-1 protein, we determined the molecular lesions of vab-1 mutant DNAs (Table 1). Several of the strong alleles had lesions consistent with their genetic behavior as null mutations. Three strong alleles, dx34, dx31, and e2027, cause deletions of sequences encoding parts of the extracellular domain (Figure 3A), and e1059 is an amber stop in the signal peptide. One strong and two intermediate alleles cause missense alterations in the extracellular...
domain and might define functionally important residues in Eph receptors. The strong allele ju8 and the intermediate allele e699 affect residues (E62K and T63I, respectively) in the N-terminal globular domain, and the e856 mutation affects the cysteine-rich domain (Figure 3D); these domains have been implicated in ephrin-Eph receptor interactions (Labrador et al., 1997).

**Mutations in the VAB-1 Kinase Domain Cause Weak Mutant Phenotypes**

Seven vab-1 mutations disrupt the kinase domain of VAB-1 (Figure 3E) and are likely to cause severe loss of kinase activity (see Discussion). Strikingly, six of these seven alleles cause weak mutant phenotypes. The weak alleles e2, e1063, and ju22 cause missense alterations of conserved residues that function to stabilize the structure of other kinase domains (Hubbard et al., 1994). Three weak alleles should truncate the kinase domain: ju63 and e116 cause stop codons, and e118 is a deletion of the C-terminal 202 residues of VAB-1. One allele, tn2, causes a stop codon in kinase subdomain IX yet causes an intermediate mutant phenotype; it is unclear why the tn2 phenotype is stronger than those of the other kinase domain alleles. The above mutations would be predicted to abolish catalytic activity of VAB-1, yet none appears to cause complete loss of vab-1 function, suggesting that VAB-1 may possess both kinase-dependent and kinase-independent functions.
vab-1 Reporter Constructs Are Widely Expressed in Early Embryos but Only in Nonepidermal Cells during Ventral Enclosure

To determine the pattern of expression of vab-1, we used reporter constructs containing translational fusions of the vab-1 locus to green fluorescent protein (GFP; see Experimental Procedures). Such vab-1::GFP constructs fully rescued vab-1(e2027) mutant phenotypes, indicating that these constructs reflect the endogenous vab-1 expression pattern. Expression from one such construct, juls24, was analyzed in detail.

VAB-1::GFP was expressed in many cells during late gastrulation; based on their positions these cells include the neuroblasts whose movement is defective in vab-1 mutants (Figure 4A) and may also include some epidermal precursors. During ventral enclosure of the epidermis, VAB-1::GFP was expressed in clusters of cells of the head and tail regions (Figures 4B–4F). In the head region, VAB-1::GFP was expressed in clusters of presumptive neuronal cells. Early in enclosure these cells appear to lie beneath the epidermal leading cells (Figures 4C and 4D); later in enclosure, the VAB-1-expressing cells lie anterior to the leading cells (Figure 4E). VAB-1::GFP was not detectably expressed in the epidermal leading cells at any stage during ventral enclosure. In the posterior of the embryo, VAB-1::GFP was expressed in several cells, including QV5 and the ventral hyp7 cells posterior to the rectum (data not shown); VAB-1::GFP was also expressed in several pharyngeal cells (Figure 4G). In late embryogenesis and throughout larval and adult development, VAB-1::GFP was localized to the axons of many neurons throughout the nervous system (Figures 4G and 4H). Thus, in most stages following gastrulation, VAB-1::GFP is widely expressed in the developing nervous system. Expression of VAB-1::GFP in the larval nervous system suggested that vab-1 might function in neural development; although vab-1 mutants do not display obvious behavioral defects, we have found that vab-1 mutants display defects in axonal outgrowth and fasciculation (S. E. G. and A. D. C., unpublished data).

vab-1 Function Is Required in Nonepidermal Cells for Epidermal Morphogenesis

The expression of VAB-1::GFP reporter constructs in nonepidermal cells during epidermal enclosure led us to ask whether the epidermal morphogenetic defects in vab-1 mutants are due to a requirement for vab-1 function in neurons. We used genetic mosaic analysis (Herman, 1995) to determine in which cells vab-1 is necessary for normal morphogenesis. Because genetic mosaics could not be identified during embryogenesis, we analyzed viable vab-1 genetic mosaics. We identified 83 vab-1 genetic mosaics, 56 of which displayed morphogenetic defects in head epidermis (the Vab phenotype). All such Vab mosaics proved to have lost vab-1 within the AB lineage, which generates head epidermal
Figure 4. Expression Pattern of vab-1 Reporter Constructs
Confocal images of VAB-1::GFP expression from the juIs24 transgene (visualized using anti-GFP antibodies [green]) and epidermal cell junctions (MH27 antigen [red]). Bars, 15 μm. (A) VAB-1::GFP expression in postgastrulation embryo (~200–250 min). Ventral view. Not all VAB-1-expressing cells have been identified at this stage; based on position most of the cells are neuroblasts. Some epidermal precursors may also express VAB-1::GFP. (B and C) Beginning of ventral closure, ventrolateral and ventral views (D). Later in ventral closure, leading epidermal cells are marked (arrows). (E) Midventral closure; leading cells have met at ventral midline, ventral view; ventral pocket has not yet closed. (F) Completed ventral enclosure (comma stage). (G) L1 larval animal showing expression of VAB-1::GFP (juIs33 transgene) in the nervous system; nerve ring and ventral nerve cord are arrowed. Strong expression is also seen in the procorpus and terminal bulb of the pharynx. (H) Expression of VAB-1::GFP in axons in late larval animal; lateral view, close-up of expression in D neuron axon commissure (arrowed) and ventral cord.
We show here that morphogenesis in *C. elegans* suggests that *vab-1* function caused strong morphogenetic defects, suggesting that *vab-1* functions in neuronal cells to regulate normal morphogenesis of the epidermis.

**Evolutionary Conservation of Eph Signaling Pathways**

*VAB-1* is most similar to receptor tyrosine kinases of the Eph subfamily. Eph receptors were first isolated from vertebrates by homology in the kinase domain (Hirai et al., 1987) and are the largest subfamily of RPTK (Orioli and Klein, 1997). Previously, Eph receptors have only been reported from vertebrates. Our findings show that the Eph receptor family is ancient and likely to be conserved among all animals.

Eph receptors bind membrane-bound protein ligands, recently renamed ephrins (Eph Nomenclature Committee, 1997). Ephrins are either membrane-anchored in cell membranes via glycosylphosphatidylinositol (GPI) anchors (ephrin-As) or are integral membrane proteins (ephrin-Bs). Eph receptors can be grouped into two subclasses: the EphA receptors, which bind ephrin-A ligands, and the EphB receptors, which bind transmembrane ephrin-B ligands (Gale et al., 1996). *VAB-1* is equally similar in sequence to EphA and EphB receptors and thus may be similar to a common ancestor of the two vertebrate subclasses. We have also identified ephrin-related genes in the *C. elegans* genomic sequence (A. D. C. and S. E. G., unpublished data), indicating that potential ligands for *VAB-1* exist in *C. elegans* and thus that ephrin signaling pathways may be conserved between nematodes and vertebrates.

**VAB-1 Might Participate in Forward and Reverse Signaling**

Vertebrate Eph receptors may participate in both kinase-dependent “forward” signaling and in kinase-independent “reverse” signaling. For example, deletion of the kinase domain of murine Nuk/EphB2 did not affect its function in axonal guidance in the anterior commissure (Henkemeyer et al., 1996). EphB2 is not expressed on anterior commissure axons but on substrate cells over which the axons navigate. A transmembrane ligand for EphB2, ephrin-B1/LEK-2, is expressed on anterior commissure axons, and EphB2 binding to ephrin-Bs can induce phosphorylation of tyrosines on the intracellular domain of the ephrin-B, thus potentially activating a signaling cascade in the ligand-expressing cell (Holland et al., 1996; Brückner et al., 1997).

**Discussion**

We show here that *vab-1*, which functions in epidermal morphogenesis in *C. elegans*, encodes an Eph receptor tyrosine kinase. The phenotypes of null and kinase domain mutations in *VAB-1* suggest that the *VAB-1* RTK has both kinase-dependent and kinase-independent functions. Our analysis of *vab-1* mutant phenotypes, expression pattern, and genetic mosaics suggests that *vab-1* functions in neuronal cells to regulate normal morphogenesis of the epidermis.
cause complete loss of activity of the LET-23 kinase (Aroian et al., 1994). Thus, the VAB-1 kinase domain mutations should abolish kinase activity.

An alternative explanation for the weak phenotypes of vab-1 kinase mutants is that VAB-1, in addition to kinase-dependent forward signaling functions, has kinase-independent reverse signaling functions. Such functions presumably require the VAB-1 extracellular domain, possibly interacting with ephrin ligands, and would explain why only mutations disrupting the VAB-1 extracellular domain would cause null phenotypes. As discussed below, VAB-1 reverse signaling could account for the nonautonomous role of vab-1 in epidermal morphogenesis. As both weak and strong vab-1 alleles appear to cause similar ranges of phenotypes with different penetrances, the kinase-dependent and kinase-independent functions of vab-1 may be required for related aspects of morphogenesis.

The Role of VAB-1 in Signaling from Neurons to Epidermis during Epidermal Morphogenesis

vab-1 mutants are defective in the movements of neuroblasts that close the ventral gastrulation cleft and the movements of the epidermal leading cells that initiate epidermal enclosure. Unexpectedly, vab-1 is not expressed in the epidermal leading cells but in neurons underlying or adjacent to the leading cells. In addition, our mosaic analysis has shown that loss of vab-1 function in nonepidermal precursors causes epidermal morphogenetic defects. Our results suggest that a major function of vab-1 is in neuronal cells and that the epidermal morphogenesis defects of vab-1 mutants in part result from defects in neuronal cells. Thus, despite its similarity to receptors, VAB-1 acts in signaling rather than responding cells.

Our observations raise two questions: what signals do underlying neurons provide to migrating epidermal cells, and does VAB-1 function directly in such neuronal-to-epidermal signaling? We propose two models for this process (Figure 6). These two models are not mutually exclusive, and the phenotypes of vab-1 mutants suggest that both models might apply. In the “steric hindrance” model (Figure 6A), VAB-1 signaling only occurs between neuronal precursors, and the epidermal defects in vab-1 mutants are a result of defects in neuronal precursors that normally provide a permissive substrate for epidermal cell movements. Mispositioned neuronal precursors resulting from the gastrulation cleft defects seen in vab-1 mutant embryos could interfere with normal epidermal migration and likely contribute to the epidermal defects seen in severely mutant vab-1 animals (class I-III). However, we often observe defects in enclosure in the absence of obvious defects in cleft closure (class IV phenotype). Thus, defects in epidermal enclosure do not appear to be solely due to defects in neuroblast movements following gastrulation.

In the “reverse signal” model (Figure 6B), neurons signal directly to epidermal cells, potentially providing cues for their migration. As discussed above, VAB-1 could participate directly in such reverse signaling, analogous to that observed for vertebrate Eph receptors. Alternatively, VAB-1 could receive a signal from epidermal cells and thereby activate a second signaling pathway in the reverse direction. Two observations further suggest that VAB-1-expressing cells might provide an inhibitory signal to epidermal cells. First, in wild-type embryos the leading cells migrate posteriorly and adja cently to VAB-1-expressing cells (Figures 4B-4E). Second, in some vab-1 mutant embryos the leading cells migrate anteriorly to their normal positions, possibly as a result of a lack of anterior repulsive cues. Identification and localization of ligands for VAB-1 is necessary to distinguish between these two models for VAB-1.

Our genetic analysis of vab-1 has shown that the null phenotype of vab-1 is a variable defect in epidermal morphogenesis. As a small percentage of vab-1 null mutants develop into apparently normal adults, vab-1 function is not essential. vab-1 signaling may be partly redundant with other signaling pathways, as found for some vertebrate Eph receptors (Orioli et al., 1996), although no additional Eph receptors have yet been identified in the C. elegans genomic sequence.

Eph Signaling in Vertebrate Morphogenesis

Signaling via Eph receptors and ligands has been implicated in axon guidance and topographic mapping (Cheng et al., 1995; Gao et al., 1996; Henkemeyer et al., 1996; Nakamoto et al., 1996). Ephrin signaling can function as a repulsive cue for axon guidance by promoting growth cone collapse (Drescher et al., 1995). Ephrin signaling has also been shown to promote axon fasciculation (Winslow et al., 1995), formation of rhombomere boundaries (Xu et al., 1995), and to inhibit cell-cell adhesion (Winning et al., 1996). A common feature of Eph-mediated signaling is thus the modulation of cell shape and cell adhesion, processes critical to epithelial morphogenesis.

The expression patterns of many vertebrate Eph receptors suggest they may be functioning in epithelial morphogenesis. For example, EphB2/Nuk is expressed in midline epithelial cells of the palatal shelves as they begin to fuse in the midline. Mice lacking both the EphB2 and EphB3/Sek4 receptors have cleft palates as a result of failure in closure of the secondary palate, possibly due to defective epithelial morphogenesis (Orioli et al., 1996). Many other Eph receptors are expressed in epithelial or endothelial organs undergoing morphogenesis, such as lung, heart (Ruiz et al., 1994), or in migrating cells (Brändli and Kirschner, 1995). Determining the
roles of vertebrate Eph receptors in these processes may be complicated by redundancy between members of this gene family; for example, EphA2/Eck is specifically expressed during mouse gastrulation (Rizzi and Robertson, 1994), yet Eck mutant mice show no discernible phenotype (Chen et al., 1996). vab-1 may provide a relatively simple model for understanding the functions of Eph receptors in these aspects of vertebrate morphogenesis.

Experimental Procedures

Genetic Analysis of vab-1

C. elegans strains were cultured using standard methods (Brenner, 1974). Mutations used were as follows: LGI, unc-29(e1072); LG II, lin-31(n301), htl-1(cc450), dpy-25(e2911dx3d), and tra-2(q122dm) (Schedl and Kimble, 1988); LG III, ncl-1(e1865); and LGX, lin-15(n765sa). Rearrangements used were ccDf4II and maDf4II. Mutations are described in Hodgkin (1997) or in references above.

vab-1 alleles were isolated in general screens for visible mutations by S. Brenner (e2, e116, e118, e200, e699, e721, e856, and e1059), J. Lewis (e1029), J. Hodgkin (e1063), A. Fire (e2027), E. Lambie (dx14, dx31), M. Zhen (ju6), D. Óstertag (ju22, ju63), and D. Greenstein (hn2). All alleles were EMS-induced except dx14 and dx31, which were UV-induced, and e2027, which arose spontaneously. All mutations fail to complement vab-1(e2). Map data showing that vab-1 lies close to the right of hlh-1 are available from the Caenorhabditis Genetics Center.

Phenotypic Analysis

We determined the penetrance of vab-1 mutant phenotypes by picking L4 animals from homzygous strains to separate plates, allowing them to self, and transferring them every 24 hr. Eggs unhatched after 24 hr were scored as embryonic arrest. Larvae that failed to develop into adults after 48 hr were scored as larval arrest. Any morphological abnormality of the head region was scored as abnormal. At least 500 individuals were scored for each genotype.

We determined the stages at which vab-1 embryos arrest by following the development of vab-1 embryos using Nomarski microscopy. Embryos were followed from comma stage or before until either development arrested or the embryo hatched. To generate translational fusion of GFP to a cDNA clone, yk18c8, contains a 2992 bp insert that we sequenced, corresponding to bases 700-3962 of the composite vab-1 cDNA. We determined the 5′ end (bases 1-699) of the vab-1 transcript in RT-PCR experiments; RT-PCR using the SL1 trans-spliced leader sequence as upstream primer (Krause and Hirsh, 1987) generated products, indicating that the vab-1 message is trans-spliced to SL1. The vab-1 cDNA sequence is 3962 bp in length, consistent with the 4 kb band observed on Northern blots (data not shown), including an SL1 trans-spliced leader, an 89 bp 5′ UTR, a 3354 bp open reading frame, and a 458 bp 3′ UTR. No evidence was found for alternative vab-1 transcripts either by Northern blot or RT-PCR experiments.

Determination of Mutant DNA Sequences

We determined the sequences of genomic DNAs from vab-1 mutants as described previously (Chisholm and Horvitz, 1995). vab-1 exons and splice sites were amplified from all vab-1 mutants (except the deletion alleles dx14, dx31, e118, and e2027) and PCR products sequenced using 32P labeled primers and the fmol kit (Promega). All mutations were confirmed on both strands and in independent PCRs. The molecular lesions of two vab-1 mutations (e721 and e1029) have not yet been found. Sequences of primers used are available upon request.

vab-1::GFP Reporter Constructs

The VAB-1::GFP construct pCZ55 used for expression studies is a translational fusion of GFP to a vab-1 minigene and contains 4.2 kb of genomic sequence 5′ to the vab-1 start codon, exons 1-5 (to the Smal site in exon 5) as genomic DNA, exons 6-10 as cDNA, GFP inserted in frame at the Xhol site near the VAB-1 N terminus, the vab-1 3′ UTR, and 0.4 kb of genomic DNA 3′ to the polyadenylation site.

Transgenic lines were generated by transformation of vab-1(e2027); lin-15 animals with pCZ55 and the lin-15 rescuing plasmid pLin-15EK (Clark et al., 1994); four independent chromosomal integrants (juls24, juls31, juls32, and juls33) were identified following X-ray mutagenesis. Expression was analyzed in the strain C2723 of genotype vab-1(e2027); lin-15(n765); juls24(vab-1::GFP); lin-15(+). Expression was analyzed by transformation of 4×GFP) by staining fixed transgenic animals with anti-GFP antibodies. Embryos were fixed in 1% paraformaldehyde and incubated with anti-GFP polyclonal antisera (Clontech) at 1:100 to 1:500 dilution and fluorescein- and TRITC-conjugated secondary antibodies using a confocal microscope. VAB-1::GFP staining patterns in all four lines were indistinguishable.

Analysis of vab-1 Genetic Mosaics

We used two approaches to identify vab-1 mosaic animals. In both approaches we generated transgenic arrays bearing wild-type copies of vab-1 and cell-autonomous marker genes (Herman, 1995). First, we generated strains of genotype unc-29; vab-1; juE[unc-29(+) vab-1(+)] sur-5(GFP) by transformation of unc-29; vab-1 worms with cosmids DNAs C45G10 (unc-29(+) [Miller et al., 1996], M03A1 (vab-1(+)) and the sur-5-GFP plasmid pTG96.1. sur-5-GFP is expressed in most somatic cell nuclei (T. Gu and M. Han, personal communication) and is a cell-autonomous marker for the array; unc-29(+) is required in body muscles (derived from P).
From such strains, three Unc non-Vab mosaics were identified and found to have lost the array in \(P_r\). We then screened for Vab non-Unc mosaics (putative mosaic mutants with losses in \(AB\)). Twenty-three of such animals were found that had patterns of sur-5-GFP interpretable as resulting from losses of the array within \(AB\).

We used the cell-autonomous marker ncl-1 (Hedgecock and Herman, 1995) in additional mosaic analysis experiments. We generated the strain C2712 vab-1, ncl-1; juc39(Jab-1 [+] ncl-1 [+] roI-6[dm]) by transformation with cosmid containing the wild-type copies of vab-1 (M03A1), ncl-1(JC33D3) [Miller et al., 1996] and pRF4. Expression of roI-6(dm) in cells contributing to the epidermal syncytium expl 7 confers a rol phenotype. Transgenic animals are thus Rol non-Vab non-Ncl. We screened C2712 Rols for rare Rol Vab animals, predicted to be mosaicics in which the array had been lost from a subset of expl 7 precursors. We found 15 mosaics with patterns of Ncl cells resulting from single losses of the array. Ncl cannot be scored in syncytial nuclei unless all precursors of the syncytium have lost the array, so we assessed the loss point by scoring identifiable cell nuclei, as described (Clark et al., 1993).

Of the 15 Rol Vab mosaic animals, 4 had losses in the nonepidermal precursors AB*np and AB*ip. As these mosaics had been selected on the basis of their Vab phenotype, it was possible that they were rare double loss mosaics in which the Vab phenotype was due to loss of the array in epidermal cells (which we could not directly score), rather than the observed loss in nonepidermal precursors.

To address this caveat we screened C2712 Rols directly for mosaicism of the Ncl marker. From 800 Rolos screened we identified 42 animals with mosaic Ncl expression. Of these 42 mosaics, 6 had lost the array in nonepidermal precursors, of which 6 were Vab, and 13 had lost the array in epidermal precursors, of which 1 was Vab. Thus, loss of vab-1 function in nonepidermal precursors can frequently cause Vab phenotypes.

Acknowledgments

We thank the colleagues above for vab-1 mutants, the C. elegans genome consortium for cosmids and sequence data, and Yuji Kohara for vab-1 cDNAs. We thank Chuck Wilson for use of his oligo synthesizer, Dawne Shetton for oligo synthesis, Bill Sullivan for help with confocal microscopy, and Doug Kellogg for the motherfode. We thank Yishi jin for extensive advice, Mei Zhen for invaluable help, Derek Ostertag and Inessa Grinberg for help with cell lineage analysis, Andy Fire for advice, Jeff Simse for help with phallolidin staining, and members of the Chisholm, jin, and Haldi labs for discussions. We thank Cori Bargmann, Gian Garriga, Ian Chin-Sang, and John Shakes, eds. (San Diego, California: Academic Press), pp. 123–146.

References


The Genbank accession number for the vab-1 cDNA sequence is AF040269.